Host Recognition by the Specialist Endoparasitoid *Microplitis croceipes* (Hymenoptera: Braconidae): Role of Host- and Plant-Related Volatiles

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The specialist parasitoid Microplitis croceipes *Cresson can parasitize only noctuid larvae in the genera* Helicoverpa *and* Heliothis. *To be successful in their search for hosts, the ability to distinguish hosts from nonhosts feeding on the same plant is beneficial. In flight tunnel experiments, we found that prior to landing on the odor source M. croceipes were able to distinguish volatiles released from frass of host larvae* (Helicoverpa zea Boddie) *and nonhost larvae* (Spodoptera exigua Hübner *and* Spodoptera frugiperda J. E. Smith) *fed on cotton. However, an initial contact experience with frass of cotton-fed host larvae appeared to be critical for this ability. Wasps that had antennated frass of host larvae fed pinto bean diet were equally attracted to frass of host and nonhost larvae fed on pinto bean diet. In short-range walking experiments, wasps located cotton-fed host larvae faster than diet-fed larvae, regardless of their experience. Wasps that had antennated frass of cotton-fed host larvae were less attracted to cotton-fed nonhost larvae, compared to host larvae, and preferred to sting host larvae. Plant-related volatiles in host frass and larvae appear to play a major role in the successful location of host larvae.*

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INTRODUCTION

Several parasitoids and predators have been discussed as being important to biological control of larvae of the *Heliothis* and *Helicoverpa* genera. The larvae cause serious damage in crops such as corn and cotton but can be regulated to some extent by predators and parasitoids such as the host-specific larval parasitoid *Microplitis croceipes* Cresson (Lewis and Brazzel, 1968; Mueller and Phillips, 1983; Knipling and Stadelbacher, 1983; Stadelbacher *et al.*, 1984; Puterka *et al.*, 1985). For specialist parasitoids such as *M. croceipes*, host-specific cues are particularly important for host location on a damaged plant. Because a plant can be attacked by several insect species, it would be beneficial for the specialist to distinguish whether the plant is attacked by a host or a nonhost prior to landing on the plant. This would minimize the time spent searching for a host on damaged plants and therefore increase the efficiency of the parasitoid. Recent results suggests that frass volatiles play a more important role in host location for the specialist parasitoid *M. croceipes* than for the generalist *Cotesia marginiventris* Cresson (Cortesero *et al.*, 1977).

The full range of cues necessary for *M. croceipes* to locate their hosts successfully and distinguish them from nonhosts on the same plant is still not completely understood. Host-produced kairomones and synomones produced by host plants are involved in attracting parasitoids and predators to the vicinity of a host (Elzen *et al.*, 1987; Drost *et al.*, 1988; Dicke and Sabelis, 1988; Dicke *et al.*, 1990a,b; Turlings *et al.*, 1991a,b; Takabayashi *et al.*, 1991; McCall *et al.*, 1993). Upon attack by herbivores several plant species release volatile compounds that appear to be specifically induced in response to herbivore damage (Turlings *et al.*, 1990; Dicke *et al.*, 1990a; McCall *et al.*, 1994; Loughrin *et al.*, 1994). These inducible compounds that are released in response to herbivore damage vary with the plant species. However, the volatile compounds released from one plant species after damage by different herbivore species do not appear to differ qualitatively (Turlings *et al.*, 1993; Blaakmeer *et al.*, 1994). After herbivore damage, inducible compounds are released from the damaged leaves and systemically throughout the entire plant (Röse *et al.*, 1996) but are not released in significant amounts from plants that are only artificially damaged with a razor blade (Turlings *et al.*, 1990; Röse *et al.*, 1996). Therefore, the release of inducible volatiles by the plant clearly signals a herbivore damaged site but may not provide the parasitoid with sufficient information about the species that attacks the plant.

Besides the strong long-range attraction of parasitoids to plant-released volatiles, many species of parasitoids are known to be attracted to frass of their host larvae (Elzen *et al.* 1987; Eller *et al.*, 1988; Turlings *et al.* 1991b; Steinberg *et al.*, 1993). Few studies address whether the specificity of the host-frass volatiles depends on the diet of the larvae (Ding *et al.*, 1989) and whether those

parasitoids might also be attracted to frass volatiles released by nonhost larvae. Wäckers and Lewis (1994) showed that *M. croceipes* can distinguish frass volatiles of larvae feeding on flowers or leaves of cotton plants. Therefore, host frass may provide specialist parasitoids with the necessary host specific cues. In addition to volatile cues, frass contains a contact kairomone that appears to be host specific (Alborn *et al.* 1995). Antennation of the contact kairomone can modify and improve the response of parasitoids to inherently attractive odors (Vet *et al.*, 1990) and promotes associative learning of novel volatile cues (Lewis and Tumlinson, 1988). Thus, antennation of host frass can confirm that the frass was produced by a host larva and *M. croceipes* wasps will search for a host in the proximity of the frass and the damaged site. This ability to recognize a host without antennation of the larvae is beneficial for a parasitoid, because corn earworm (CEW), *Helicoverpa zea* Boddie, larvae will very aggressively defend themselves. Attacked larvae may bite and seriously injure a wasp. In addition, larvae may regurgitate on the wasp, which forces the parasitoid to clean itself, giving the host time to escape. Therefore, it would benefit the wasp to use volatile cues released by the larva to identify and locate the host after antennating the frass, but without antennating the larva prior to stinging.

The nonspecificity of plant volatiles released after feeding damage of different herbivore species led us to examine frass and larvae as possible sources of host-specific volatile cues for the specialist parasitoid *M. croceipes*. We further investigated how these cues are affected by the diet of the larva. In flight tunnel experiments, we examined the host specificity of volatiles released from frass of different lepidopteran species feeding on cotton and whether the ability to distinguish is affected by preflight experience of the wasps and the diet of the larvae producing the frass. In short-range walking experiments we investigated whether the ability to find and accept a larva for parasitization depends on previous frass experience and on the diet of the larvae releasing those volatiles.

MATERIALS AND METHODS

Plants

Cotton plants, *Gossypium hirsutum* L. (cv. Deltapine acala 90), were grown in 16-cm-diameter pots filled with a potting soil and vermiculite mixture (3:1) in a greenhouse. The greenhouse was illuminated with natural light, and conditions were ambient for Florida summer (14L:10D light cycle, 85 ± 10% relatively humidity, and 30 ± 10°C). Plants were fertilized once at time of planting with a 3- to 4-month formulation of Osmocote 14-14-14 (N-P-K) controlled-release fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH). Larvae were fed plants that were about 5 weeks old and had six fully developed leaves in addition to the two cotyledons.

Lepidoptera Larvae

Beet armyworm larvae (BAW), *Spodoptera exigua* Hübner, fall armyworm larvae (FAW), *Spodoptera frugiperda* J. E. Smith, and corn earworm larvae (CEW), *Helicoverpa zea* Boddie, were obtained from the Insect Attractants, Behavior, and Basic Biology Research Laboratory, Gainesville, Florida. Larvae were reared according to the method of King and Leppla (1984), on an artificial diet, based on pinto beans. Third- to fourth-instar larvae of each species were used for the frass collections and for petri dish bioassays.

Parasitoids

The specialist larval endoparasitoid *M. croceipes* was reared from cocoons obtained from a colony maintained at the U.S. Department of Agriculture—Agricultural Research Service, Insect Biology and Population Management Research Laboratory, Tifton, GA. Parasitoids were reared on larvae of CEW fed on CSM (Blended Food Product, Child Food Supplement, Formula No. 2) diet (Burton, 1970) as described by Lewis and Burton (1970). Cocoons were separated from hosts prior to emergence of adult wasps and female and male parasitoids were kept together in screen cages (25 × 25 × 25 cm) in the laboratory to allow mating. Parasitoids were kept in the laboratory at 14-h photophase, a temperature of 25 ± 5°C, and 60 ± 5% RH and were fed with honey and water after emergence. Mated females used for flight tunnel experiments were 3–4 days old and were transferred to the flight tunnel room 3 h prior to the experiment to adjust to the flight tunnel conditions. All females were used only once in an experiment.

Frass Collection

Frass was collected from larvae that had been caged with cotton leaves for 48 h. To avoid inclusion of plant particles in the frass, larvae were transferred at 0900 to a clean multicellular tray with a separate compartment for each larva. The tray was covered with wet paper towels to maintain high humidity, and after 2 h frass excreted during this time was collected. Frass from diet fed larvae was collected in the same manner. For flight tunnel tests, 20 mg of freshly collected frass was placed in a glass tube, open at both ends (10 cm long × 0.5-cm in outside diameter) used as an odor release device.

Preflight Experience

For all no-choice experiments wasps were allowed to antennate CEW host frass (three times for 30 s, with a 1-min interval between experiences). Host frass used for preflight experiences was freshly (less than 1 h old) excreted by cotton-fed host larvae.

For two-choice experiments (Table I) parasitoids were given one of four

Table I. Preflight Experiences and Odor Sources in the Flight Tunnel for Different Two-Choice Experiments[a]

Experiment	Preflight experience	Odor sources in flight tunnel	Number of wasps tested
A	Frass of cotton-fed CEW	Frass of cotton-fed CEW vs. BAW CEW vs. FAW	100
B	Frass of diet-fed CEW	Frass of diet-fed CEW vs. BAW CEW vs. FAW	140
C	Naive	Frass of cotton-fed CEW vs. BAW CEW vs. FAW	100
D	Atennate and parasitize 3rd-instar diet-fed CEW	Frass of cotton-fed CEW vs. BAW CEW vs. FAW	100
E	Frass of diet-fed CEW	Frass of cotton-fed CEW vs. BAW CEW vs. FAW	100
F	Frass of cotton-fed CEW	Frass of cotton-fed CEW + BAW vs. CEW	50

[a] All experiments were conducted comparing host frass odor from corn earworm larvae (CEW) with nonhost frass odor from beet armyworm larvae (BAW), and in a second two-choice experiment comparing host frass odor (CEW) with nonhost frass odor from fall armyworm larvae (FAW).

preflight experiences immediately prior to the release in the flight tunnel. (1) Wasps were allowed to antennate (three times for 30 s, with a 1-min interval between experiences) freshly excreted CEW host frass from cotton-fed larvae (experiment A and F). (2) Wasps were allowed to antennate freshly excreted frass from diet-fed host larvae (experiments B and E). (3) Wasps were allowed to parasitize and antennate a third-instar CEW larve fed on diet (experiment D). Wasps that were injured or regurgitated upon by the larvae were discarded. (4) No preflight conditioning was given to wasps, referred to as naive (experiment C).

Flight Tunnel Experiments

All free flight experiments with *M. croceipes* were carried out in a Plexiglas flight tunnel, 60 × 60 cm in cross section and 240 cm long, with an airflow of 0.2 m/s. Four Krypton lights (90 W) illuminated the flight tunnel with approximately 800 lux from above. Details of this tunnel have been described by Eller *et al.* (1988) and Turlings *et al.* (1991a). A temperature of 27 ± 1°C and 75 ± 5% RH were maintained in the flight tunnel during the experiments. All experiments were conducted 3–5 h into the photophase, between 1030 and 1230.

Odors were released into the flight tunnel by blowing humidified air, at a rate of 100 ml/min, over each odor source held in a glass tube (described in frass collection above). Odor sources were held parallel with the air flow, 25 cm above the floor of the flight tunnel (separated by 12 cm for the two-choice experiments) and equidistant from the parasitoid release point. Parasitoids were released individually in a glass cylinder 25 cm above the floor and 80 cm downwind of the odor sources. The glass cylinder ended in a curved funnel, opening into a glass tube (Turlings et al., 1991b) that was oriented parallel to the air flow. The odors released upwind passed through the glass tube, which prevented the insects from taking flight before detecting the odor sources.

In all bioassays, parasitoids were given three chances to complete a flight by landing on an odor source after a nonstop flight. After an incomplete flight, the parasitoid was returned to the release chamber. The position of the two odor sources in the flight tunnel was switched routinely after each completed flight, to avoid positional bias. For non-choice experiments, the number of completed and noncompleted flights of parasitoids to frass of cotton-fed CEW, BAW, and FAW was recorded. For two-choice experiments, the choice of the parasitoid after a completed flight was recorded, as well as the number of wasps that did not complete flights. Two-choice experiments were carried out in the flight tunnel comparing host frass odor (20 mg of CEW frass) with a nonhost frass odor (20 mg of BAW or FAW frass) for experiments A, B, C, D, and E. For experiment F (Table I), odor released by 40 mg of frass from cotton-fed CEW was compared to odor released by a mixture of 20 mg of frass of cotton-fed BAW and 20 mg of frass of cotton-fed CEW. Frass used as an odor source was collected from larvae fed on cotton plants (Table I, experiments A, C, D, E, F) or from larvae fed on diet (Table I, experiment B).

Each no-choice test to volatiles of frass of cotton-fed BAW, FAW or CEW was conducted on five separate days with a total of $n = 50$ wasps tested for their attraction to frass volatiles of each caterpillar species. Differences in the total number of wasps that completed flights to frass volatiles of each larval species were analyzed by a chi-square test (SYSTAT, Systat Inc., Evanston, IL). Each two-choice experiment to volatiles of host frass compared to nonhost BAW frass and host frass to nonhost FAW frass was conducted on five or more separate days. A total of $n = 100$ wasps was tested in each experiment A, C, D, and E, a total of $n = 140$ wasps was tested in experiment D, and $n = 50$ wasps were tested in experiment F for each two-choice combination of host frass compared to nonhost frass. Differences in the numbers of wasps that made a choice between volatiles from host or nonhost frass were analyzed by a chi-square test. Differences in the total number of completed flights to frass of cotton-fed larvae after different preflight experiences or no preflight experience were compared by a chi-square test. Comparisons yielding a p value ≤ 0.05 were considered to be statistically significant in all experiments.

Table II. Experience and Type of Larvae Encountered by Female *M. croceipes* Wasps in No-Choice Petri Dish Experiments

Experiment	Experience	Larvae encountered	Number of wasps tested
G	Frass of cotton-fed CEW	Cotton-fed CEW	10
	Frass of diet-fed CEW	Cotton-fed CEW	
		Diet-fed CEW	
	Naive	Cotton-fed CEW	
		Diet-fed CEW	
H	Frass of cotton-fed CEW	Cotton-fed BAW	20
		Cotton-fed FAW	
		Cotton-fed CEW	

Experience Prior to Petri Dish Experiments

With no-choice petri dish experiments, we investigated (Table II, experiment G) (1) whether the diet of a host larva would affect *M. croceipes'* ability to locate the larvae, (2) the effect of a prior frass experience on the wasp's ability to locate larvae, and (3) whether the wasp would indiscriminately sting any host or nonhost larva it encountered after antennating frass of host larvae (CEW) (Table II, experiment H).

A 1.5-cm-ID circular opening was cut out of the center of the bottom of several petri dishes. To give a wasp a frass antennation experience, 2 mg of freshly produced CEW frass was rubbed over the edge of the hole of a petri dish. A vial containing the wasp was then placed under the opening of the dish. This ensured an encounter of frass by the wasp when leaving the vial. Wasps were allowed to antennate the frass three times for 30 s, with a 1-min interval between experiences, and then returned to the vial between experiences.

Petri Dish Experiments

Within 30 s after the frass experience, the vial containing the wasp was placed under a new petri dish containing a larva of one of the three insect species. All third to fourth instar larvae selected for bioassays were of the same size and most larvae walked around the outer edge of the petri dish. By using separate petri dishes for the frass experience and the larva encounter, we avoided interference of frass volatiles with the wasp search for a larva. Observations were recorded using "The Observer" (Version 3.0, Noldus Information Technologies, Wageningen, The Netherlands).

In a pilot test, wasps that did not find a larva within 5 min did not find it within 15 min either. In subsequent tests all wasps were allowed up to 8 min for searching. Wasps that found the larvae within 8 min were recorded as an encounter, and the time to the encounter was recorded. Wasps that had encoun-

A *experience on CEW cotton frass - flight to cotton frass*

complete flights (%)

| * BAW | 20 | 42 | CEW | 62 |
| * FAW | 13 | 39 | CEW | 52 |

B *experience on CEW diet frass - flight to diet frass*

| BAW | 40 | 34 | CEW | 53 |
| FAW | 31 | 49 | CEW | 57 |

C *no experience "naive" - flight to cotton frass*

| BAW | 2 | 5 | CEW | 7 |
| FAW | 3 | 4 | CEW | 7 |

D *sting of diet fed CEW - flight to cotton frass*

| BAW | 18 | 26 | CEW | 44 |
| FAW | 15 | 13 | CEW | 28 |

E *experience on CEW diet frass - flight to cotton frass*

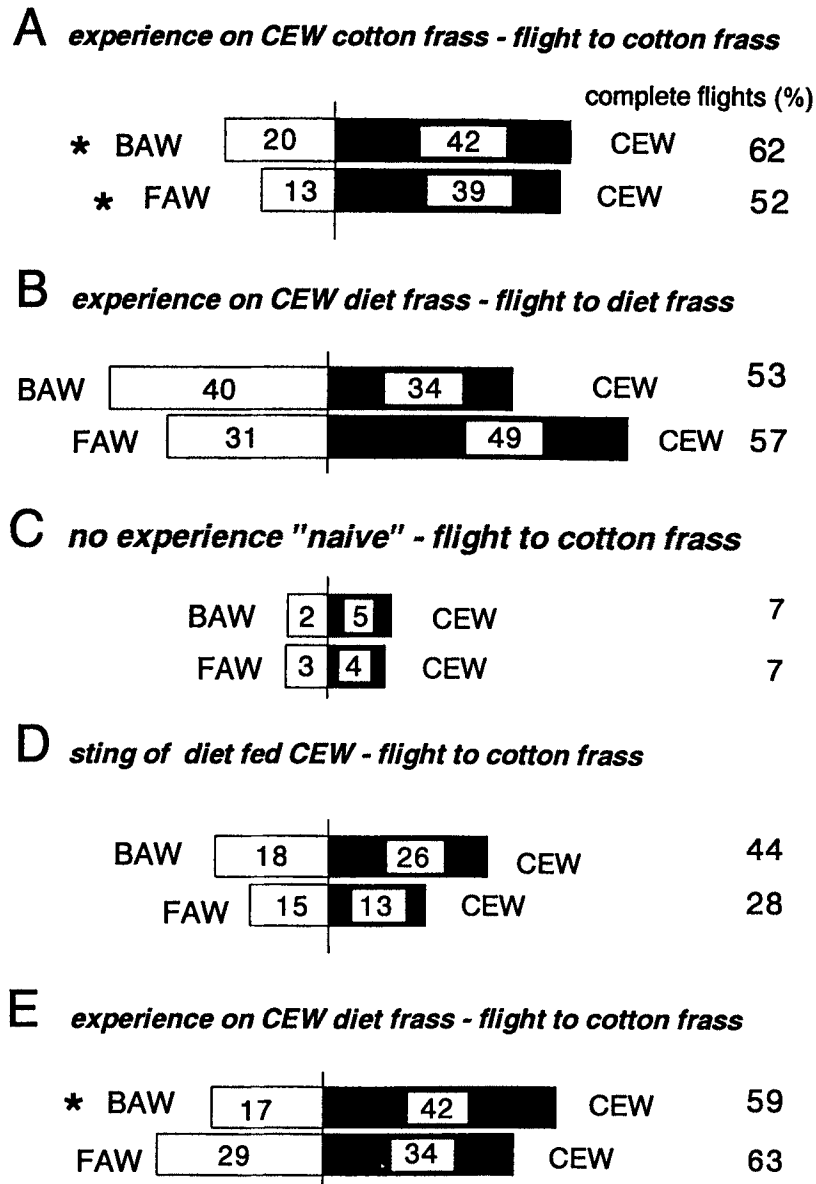| * BAW | 17 | 42 | CEW | 59 |
| FAW | 29 | 34 | CEW | 63 |

Fig. 1. Flight response of *M. croceipes* in two-choice experiments to frass volatiles of cotton-fed or artificial diet-fed larvae of the host, *H. zea* (CEW), compared to nonhost frass volatiles from *S. exigua* (BAW) or *S. frugiperda* (FAW) after different preflight experiences. Total numbers of completed flights are reported as percentages, whereas numbers in the bars represent the number of wasps that completed flights to each volatile source. Response to

tered a larva were grouped into three categories: Stinging without antennating the larvae, stinging following antennation, and rejection following an antennation of the larva.

In the first set of experiments (Table II, experiment G), wasps were given an antennation experience with frass of cotton or diet-fed CEW or had no experience. Each wasp was then presented a CEW larva that had been either cotton-fed or diet-fed. A total of 10 wasps were tested for each combination. In the second set of experiments (Table II, experiment H), wasps were given an experience with frass of cotton-fed CEW and then presented cotton-fed CEW, BAW, or FAW in the petri dish. A total of 20 wasps was tested per combination.

Differences in the number of larvae encountered by the wasps after different experiences and the number of the encountered larvae that were stung without prior antennation were tested for significance using Fisher's exact test (SYS-TAT, Systat Inc., Evanston, IL). Differences in the time to first encounter were tested for significance using the Tukey test, HSD. A P value ≤ 0.05 was considered to be statistically significant in all cases.

RESULTS

Flight Tunnel Experiments

Wasps that were given a preflight experience with frass of cotton-fed host larvae clearly responded to volatiles released by frass of cotton-fed host and nonhost larvae. In no-choice experiences wasps responded equally to frass volatiles released by frass of cotton-fed CEW (39 complete flights from 50 parasitoids), BAW (39 complete flights from 50 parasitoids), and FAW (38 complete flights from 50 parasitoids). However, in two-choice experiments, volatiles released from frass of cotton-fed CEW larvae were significantly preferred over volatiles released from frass of nonhost BAW or FAW larvae (Fig. 1, experiment A). When the parasitoids were given an experience with frass of artificial diet-fed larvae, the percentage of wasps responding to artificial diet frass volatiles was comparable to the percentage responding to cotton frass volatiles in the previous experiment (Figure 1, experiment B). However, these parasitoids were

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frass volatiles of (A) cotton-fed host and nonhost larvae after preflight experience on frass of cotton-fed CEW larvae; (B) artificial diet-fed host and nonhost larvae after preflight experience on frass of artificial diet-fed CEW larvae; (C) cotton-fed host and nonhost larvae after no preflight experience (naive), (D) cotton-fed host and nonhost larvae after a stinging experience of an artificial diet-fed CEW larvae; and (E) cotton-fed host and non-host larvae after preflight experience on frass of artificial diet-fed CEW larvae. A total of 100 wasps was tested in each experiment A, C, D, and E and 140 wasps were tested in experiment B. The total numbers of wasps responding to each two-choice flight combination of host frass and nonhost frass were analyzed by chi-square test. Comparisons yielding a value of $P \leq$ 0.005 were considered to be statistically significant and are indicated by an asterisk.

not able to distinguish volatiles released by frass of diet-fed hosts (CEW) or non-hosts (BAW or FAW) (Fig. 1B). The ability of the parasitoid to distinguish between frass volatiles of their hosts and those of nonhosts appeared to depend on the diet of the larvae.

To determine whether the ability to distinguish between frass volatiles of plant-fed host and those of plant-fed nonhost larvae was innate or learned, we conducted experiments with wasps that were inexperienced (naive) with host-, host frass-, or plant-related cues. Overall, naive wasps completed significantly fewer flights to frass volatiles from cotton-fed larvae than wasps experienced on frass of cotton-fed or diet-fed host larvae (Fig. 1, experiments A and E compared to experiment C, total number of complete flights for each two-choice combination; $P \leq 0.001$ for all comparisons of experienced wasps to naive wasps). Only 7% of the naive wasps completed a direct flight to one of the odor sources, and they did not distinguish between frass volatiles of cotton-fed host and those of plant-fed nonhost larvae (Fig. 1C). Because of the increased number of wasps responding to frass volatiles after frass experience compared to naive wasps, it appears that some host related cues were necessary to activate host-searching behavior. To increase the responsiveness of the wasps in a subsequent experiment without providing cotton frass-related cues, wasps were allowed to parasitize diet-fed host larvae (Fig. 1, experiment D). As a result of the stinging experience, those parasitoids completed overall significantly more flights to cotton frass volatiles than naive wasps (Fig. 1; completed flights experiment C compared to completed flights experiment D, $P \leq 0.001$ for BAW/CEW and $P < 0.001$ for FAW/CEW two-choice combinations) but significantly less than parasitoids that were experienced on frass of cotton- or diet-fed host larvae (Fig. 1, completed flights experiment A compared to completed flights experiment D, cotton frass experience—$P \leq 0.011$ for BAW/CEW frass volatiles, $P \leq 0.001$ for FAW/CEW frass volatiles; Fig. 1, completed flights experiment E compared to completed flights experiment D diet frass experience—$P \leq 0.034$ for BAW/CEW frass volatiles, $P \leq 0.001$ for FAW/CEW frass volatiles). Despite an increased flight response, wasps that were allowed to parasitize a diet-fed larva were not able to distinguish between host and nonhost frass volatiles in the flight tunnel (Fig. 1, experiment D). This shows that an experience with frass was necessary for the parasitoid to be able to distinguish between frass volatiles of cotton-fed host and those of cotton-fed nonhost larvae.

Subsequently, we observed whether parasitoids had to experience frass of cotton-fed host larvae to recognize their host using cotton frass volatiles or whether an experience with frass of artificial diet-fed hosts was sufficient. Wasps that had preflight experiences with artificial diet host frass completed as many flights to frass volatiles of cotton-fed larvae, as did wasps that were experienced on cotton frass (Fig. 1, experiments A and E). However, after experiencing frass of artificial diet-fed host larvae, only wasps that were given a choice

between frass volatiles of cotton-fed CEW-host and BAW-nonhost larvae significantly preferred frass volatiles of their host over the nonhost (Fig. 1, experiment E). Wasps showed no preference when given a choice of frass volatiles of cotton-fed host larvae compared to frass of FAW nonhost larvae (Fig. 1E). The ability to choose host frass volatiles over nonhost BAW frass volatiles would be due to a repellent effect of BAW frass or a lack of attraction in BAW frass volatiles rather than a host-specific attraction to CEW frass volatiles. However, experiments with 50 wasps showed that of 32 responding wasps, 15 wasps chose a mixture of BAW and CEW frass volatiles, compared to 17 wasps choosing CEW frass volatiles. Therefore, as indicated previously by no-choice experiments, volatiles of BAW frass did not appear to repel the wasps.

Petri Dish Experiments

All cotton-fed CEW larvae were found significantly faster by parasitoids than diet-fed larvae, independent of the wasp's earlier experience (Fig. 2). This indicates that the wasps were innately attracted to plant related volatiles released by the larvae. However, the willingness of the wasp to sting a larva without prior antennation seemed to depend on experience (Fig. 3). Wasps that had experienced frass of cotton or diet-fed CEW were equally willing to sting encountered cotton- and diet-fed CEW without prior antennation (Fig. 3; STG). Naive wasps were significantly less willing to sting a cotton-fed CEW without prior attennation compared to frass-experienced wasps (Fig. 3; STG). However,
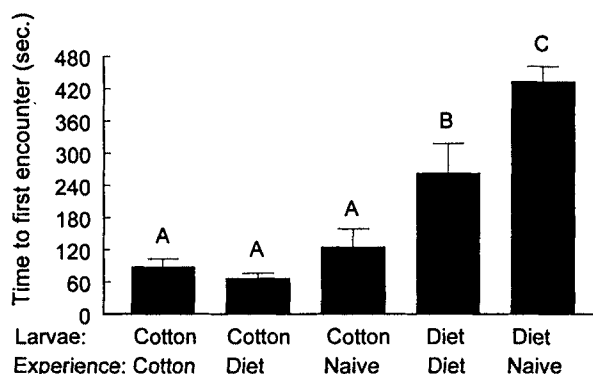

Fig. 2. Time to first encounter of host larva fed on cotton or diet by the wasps after antennation experience of frass of cotton- or diet-fed hosts or after no experience (naive). Measurement started when the wasp walked into the petri dish containing a CEW and lasted until the larva was encountered or a maximum of 480 s. Each bar represents the mean of 10 replicates with standard error. Bars topped by the same letter are not significantly different (Tukey test, HSD, $P \leq 0.05$).
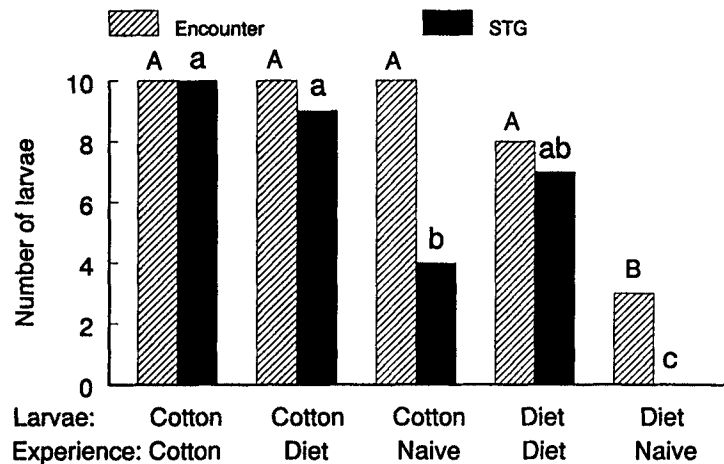
Fig. 3. Number of cotton- or diet-fed host larvae encountered and stung within 480 s by the wasps after antennation experience of frass of cotton- or diet-fed hosts or after no experience (naive) Different capital letters above bars indicate significant differences ($P \le 0.05$) between numbers of larvae encountered (encounter), and different lowercase letters indicate significant ($P \le 0.05$) differences between numbers of larvae stung without attenuation (STG; black bars), using Fisher's exact test.

all cotton-fed larvae were found by naive wasps (Fig. 3). After antennation all host larvae were stung regardless of the larval diet (not shown in Fig.). Although antennation of a larva can confirm its identity as a host by contact kairomones, the risk of a wasp to be injured by the larvae is lower, if the parasitoid spends less time antennating. Therefore, the ability of the wasp to learn to identify a host without antennation is very beneficial.

Wasps that had experienced frass of cotton-fed host larvae seemed to be able to detect if the larva presented to them in the petri dish was a host (Fig. 4). Fewer nonhost than host larvae were encountered, and this difference was significant for BAW compared to CEW. Significantly fewer BAW (80%) and FAW (47%) were stung without prior antennation compared to CEW (100%). Of the FAW that were antennated after an encounter, 78% were rejected by the wasps, making the total number of FAW stung significantly lower compared to both BAW and CEW. This indicates that *M. croceipes* might use different cues to distinguish the two nonhost BAW and FAW from the host CEW.

DISCUSSION

Our data show that in choice tests *M. croceipes* is able to distinguish between frass volatiles of cotton-fed hosts and those of cotton-fed nonhost lep-
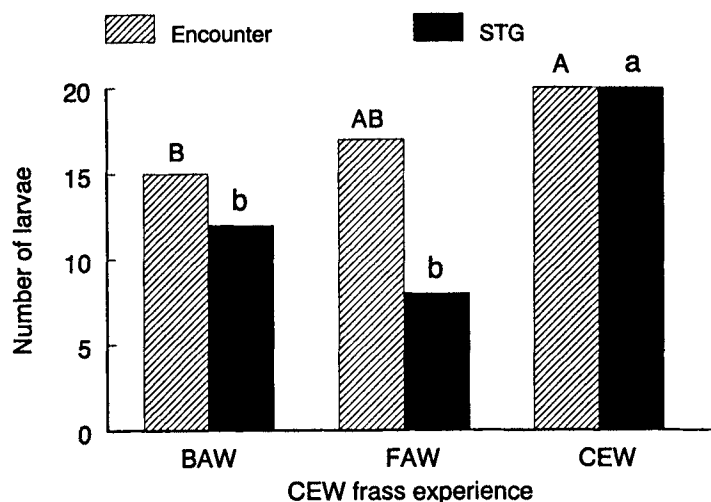
Fig. 4. The number of cotton-fed BAW, FAW, and CEW encountered and stung within 480 s by wasps after experience of frass of cotton-fed CEW. Different capital letters above bars indicates significant differences ($P \leq 0.05$) between numbers of larvae encountered (encounter), and different lowercase letters indicate significant ($P \leq 0.05$) differences between numbers of larvae stung without antennation (STG; black bars), using Fisher's exact test.

idopteran species in a flight tunnel but not between frass of larvae fed on pinto bean diet. Therefore, host frass from cotton-fed larvae is a source of volatiles that provides the parasitoid *M. croceipes* with host specific cues, detectable prior to landing on a herbivore damaged plant. Compounds originating from plants appear to be necessary for parasitoids to distinguish between host and nonhost frass prior to landing on the odor source. Plant compounds may be modified by the host larvae in a specific way or may be present in different amounts in host frass compared to nonhost frass. Evidence of chemical differences in the blend of frass volatiles released by different herbivore *Pieris* spp. feeding on cabbage was reported by Agelopoulos and Keller (1994).

Recent results indicate that naive *M. croceipes* wasps respond innately to cotton-related volatiles in frass when presented along with the plant (Cortesero *et al.* 1997). In our experiments, where only frass was presented to the wasps, naive *M. croceipes* completed significantly fewer flights to frass volatiles of cotton-fed larvae than wasps experienced on host frass. It appears that some host-related cues are necessary to activate host-searching behavior. This is in accordance with previous findings that antennation of host frass will sensitize and activate host-searching behavior in *M. croceipes* (Eller *et al.*, 1992; McCall *et al.*, 1993).

Parasitoids are able associatively to learn novel odors in conjunction with

the host and antennation of host frass (Vinson et al., 1976; Lewis and Tumlinson, 1988). In our no-choice experiments, the perception of a host-specific contact recognition kairomone in the frass (Alborn et al., 1995) enabled the parasitoid to associate host unspecific cotton volatile odors in frass with the host. Therefore, the wasps completed a similar number of flights to frass of host and nonhost larvae after experiencing frass of cotton-fed host larvae in a no-choice experiment. Similar results were reported for no-choice flight tunnel tests with females of the specialist parasitoid Cotesia rubecula Marshall. Those parasitoids responded also to frass of host and nonhost Pieris spp. reared on brussels sprouts (Agelopoulos et al. 1995).

To be able to distinguish frass volatiles of cotton-fed hosts and nonhosts in two-choice experiments, wasps had to experience host frass. When the wasps were experienced on frass from diet-fed host larvae, the wasps could distinguish only frass from cotton-fed CEW and BAW, and could not distinguish frass from cotton-fed CEW and FAW. Although wasps had not experienced frass of cotton-fed hosts, they could distinguish it from frass of cotton-fed BAW in two-choice flight tunnel experiments. Therefore, this presence may not be learned. A possible explanation would be that CEW hosts, but not BAW, converted cotton plant constituents to volatiles innately recognized by the wasps that have been primed or motivated by contact with the host recognition kairomone found in host frass. Because wasps experienced with frass of diet-fed hosts did not differentiate frass volatiles of cotton-fed FAW and CEW, we may assume they both contained the converted plant constituents innately recognized by the wasps. However, because wasps distinguished frass volatiles of cotton-fed FAW and CEW after an experience with frass of cotton-fed CEW, there appeared to be additional odors released from constituents of plant materials by CEW, but not by FAW, that were learned by the wasps. There is a further possibility that previously low response levels to specific host-produced compounds are increased when experienced in combination with certain cotton plant volatiles (Vet et al., 1990).

In our no-choice petri dish experiments, wasps that had experienced host frass encountered significantly fewer of the nonhost BAW and stung significantly fewer of the nonhost BAW and FAW than the host CEW. Therefore, in addition to attraction to nonspecific cotton volatiles and physical stimuli such as size and shape of the larvae (Vinson, 1977), it appears that after landing on a damaged site and antennation of frass, wasps can learn and utilize host-specific volatiles to find the host larva. The large number of FAW that were antennated and rejected indicates the wasps might also be able to utilize other cues to distinguish host from nonhost larvae. Interestingly, BAW is completely unacceptable as a host due to a high rate of egg encapsulation (Blumberg and Ferkovich, 1994) but, in our experiments, were more readily accepted upon contact than FAW that encapsulate parasitoid eggs at a lower rate (Blumberg and Ferkovich, 1994).

Naive *M. croceipes* wasps were more likely to encounter a cotton-fed larva than an artificial diet-fed larva. Therefore, our experiments indicate an innate response to cotton volatiles released by the larvae. Elzen *et al.* (1984) showed in a two-choice test that the parasitoid *Campoletis sonorensis* Cameron preferred cotton-fed *Heliothis virescens* over wheat germ diet-fed larvae. The parasitoids were most likely responding to cotton-related volatiles in the cuticle of the larvae (Elzen *et al.*, 1984). However, even if naive wasps in our experiments were highly attracted to cotton-fed host larvae, they usually antennated the larvae prior to stinging. Wasps that had experienced frass of artificial diet or cotton-fed CEW were more willing to sting encountered larvae than were the naive wasps, indicating the importance of a contact with the recognition kairomone present in the frass.

Our observations with regard to frass volatiles may have implication for prerelease conditioning of mass reared parasitoids as well as increasing our basic understanding of foraging strategies of parasitoids. The response to host frass volatiles appeared to be at least partially learned. Therefore, an associative learning experience of the contact kairomone in host frass with the host-modified plant comopunds appeared to be necessary to enable the parasitoid to recognize its host. Even though plant volatiles may play the more important role in long-range attraction to herbivore-damaged plant, our results show that frass volatiles are an important source of host specific cues. Cotton plants that release herbivore inducible compounds systemically (Röse *et al.*, 1996) attract specialist *M. croceipes* wasps and generalist *C. marginiventris* wasps (Röse *et al.* 1997). However, when given a choice between cotton plants that systemically released inducible compounds and undamaged cotton plants with frass applied on the leaves, the specialist *M. croceipes* preferred plants with applied frass, whereas the generalist *C. marginiventris* exhibited no clear preference (Cortesero *et al.*, 1997). As these results indicate, frass volatiles are very important in host searching behavior and may be particularly important for specialist parasitoids such as *M. croceipes* because they can provide host-specific cues in addition to induced plant compounds that are also inducible by feeding of herbivores other than their host (Röse *et al.*, 1997). Parasitoids that eclose from plant-fed hosts are likely to experience plant related cues at emergence, whereas mass-reared parasitoids may lack this experience (Hérard *et al.* 1988a,b). Therefore, mass-reared parasitoids should be provided not only with cues about their target plant but also with additional host-specific cues.

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REFERENCES

Agelopoulos, N. G., and Keller, M. (1994). Plant-natural enemy association in tritrophic system, *Cotesia rubecula—Pieris rapae*—Brassicaceae (Cruciferae). III. Collection and identification of plant and frass volatiles. *J. Chem. Ecol.* **20:** 1955–1967.

Agelopoulos, N. G., Dicke, M., and Posthumus, M. A. (1995). Role of volatile infochemicals emitted by feces of larvae in host-searching behavior of parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae): A behavioral and chemical study. *J. Chem. Ecol.* **21:** 1789–1811.

Alborn, H. T., Lewis, W. J., and Tumlinson, J. H. (1995). Host-specific recognition kairomone for the parasitoid *Microplitis croceipes* (Cresson). *J. Chem. Ecol.* **21:** 1697–1708.

Blaakmeer, A., Geervliet, J. B. F., Van Loon, J. J. A., Posthumus, M. A., Van Beek, T. A., and De Groot, A. E. V. (1994). Comparative headspace analysis of cabbage plants damaged by two species of *Pieris* caterpillars: Consequences for in-flight host location by Cotesia parasitoids. *Entomol. Exp. Appl.* **73:** 175–182.

Blumberg, D., and Ferkovich, S. M. (1994). Development and encapsulation of the endoparasitoid, *Micropolitis croceipes* (Hym.: Braconidae), in six candidate host species (Lep.). *Entomophaga* **39:** 293–302.

Burton, R. L. (1970). A low-cost artificial diet for the corn earworm. *J. Econ. Entomol.* **63:** 1969–1970.

Cortesero, A. M., De Moraes, C. M., Stapel, J. O., Tumlinson, J. H., and Lewis, W. J. (1997). Comparison and contrasts in host foraging strategies of two larval parasitoids with different degrees of host specificity *J. Chem. Ecol.* **23:**1589–1606 (1997).

Dicke, M., and Sabelis, M. W. (1988). How plants obtain predatory mites as bodyguards. *Neth. J. Zool.* **38:** 148–165.

Dicke, M., van Beek, T. A., Posthumus, M. A., Ben Dom, N., van Bokhoven, H., and de Groot, A. E. (1990a). Isolation and identification of volatile kairomone that affects acarine predator-prey interactions. *J. Chem. Ecol.* **16:** 381–396.

Dicke, M., Sabelis, M. W., Takabayashi, J., Bruin, J., and Posthumus, M. A. (1990b). Plant strategies of manipulating predator-prey interactions through allelochemicals: prospects for application in pest control. *J. Chem. Ecol.* **16:** 3091–3118.

Ding, D., Swedenborg, P. D., and Jones, R. L. (1989). Chemical stimuli in host-seeking behavior of *Macrocentrus grandii* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* **82:** 232–236.

Drost, Y. C., Lewis, W. J., and Tumlinson, J. H. (1988). Beneficial arthropod behavior mediated by airborne semiochemicals. V. Influence of rearing method, host-plant, and adult experience on the host-searching behavior of *Microplitis croceipes* (Cresson), a larval parasitoid of *Heliothis*. *J. Chem. Ecol.* **14:** 1607–1616.

Eller, F. J., Tumlinson, J. H., and Lewis, W. J. (1988). Beneficial anthropod behavior mediatd by airborne semiochemicals: Source of volatiles mediating the host-location flight behavior of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). *Environ. Entomol.* **17:** 745–753.

Eller, F. J., Tumlinson, J. H., and Lewis, W. J. (1992). Effect of host diet and preflight experience on the flight response of *Microplitis croceipes* (Cresson). *Physiol. Entomol.* **17:** 235–240.

Elzen, G. W., Williams, H. J., and Vinson, S. B. (1984). Role of diet in host selection of *Heliothis virescens* by parasitoid *Campoletis sonorensis* (Hymenoptera: Ichneumonidae). *J. Chem. Ecol.* **10:** 1535–1541.

Elzen, G. W., Williams, H. J., Vinson, S. B., and Powell, J. E. (1987). Comparative flight behavior of parasitoids *Campoletis sonorensis* and *Microplitis croceipes. Entomol. Exp. Appl.* **45:** 175–180.

Hérard, F., Keller, M. A., Lewis, W. J., and Tumlinson, J. H. (1988a). Beneficial arthropod behavior mediated by airborne semiochemicals. III. Influence of age and experience on flight chamber responses of *Microplitis demolitor* Wilkinson. *J. Chem. Ecol.* **14:** 1583–1596.

Hérard, F., Keller, M. A., Lewis, W. J., and Tumlinson, J. H. (1988b). Beneficial arthropod behavior mediated by airborne semiochemicals. IV. Influence of host diet on host-oriented flight chamber responses of *Microplitis demolitor* Wilkinson. *J. Chem. Ecol.* **14:** 1597–1606.

King, E. G., and Leppla, N. C. (1984). *Advances and Challenges in Insect Rearing*, Agriculture Research Service USDA, U.S. Government Printing Office, Washington, DC.

Knipling, E., and Stadelbacher, E. A. (1983). The rationale for areawide management of *Heliothis* (Lepidoptera: Noctuidae) populations. *Bull. Entomol. Soc. Am.* **29:** 29–37.

Lewis, W. J., and Brazzel, J. R. (1968). A three-year study of parasites of the bollworm and the tobacco budworm in Mississippi. *J. Econ. Entomol.* **61:** 673–676.

Lewis, W. J., and Burton, R. L. (1970). Rearing *Microplitis* in the laboratory with *Heliothis zea* as hosts. *J. Econ. Entomol.* **63:** 656–658.

Lewis, W. J., and Tumlinson, J. H. (1988). Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* **331:** 257–259.

Loughrin, J. H., Manukian, A., Heath, R. R., Turlings, T. C. J., and Tumlinson, J. H. (1994). Diurnal cycle of emission of induced volatile terpenoids by herbivore-injured cotton plants. *Proc. Natl. Acad. Sci. USA* **91:** 11836–11840.

McCall, P. J., Turlings, T. C. J., Lewis, W. J., and Tumlinson, J. H. (1993). Role of plant volatiles in host location by the specialist parasitoid *Microplitis croceipes* Creson (Braconidae: Hymenoptera). *J. Insect Behav.* **6:** 625–639.

McCall, P. J., Turlings, T. C. J., Loughrin, J., Proveaux, A. T., and Tumlinson, J. H. (1994). Herbivore-induced volatile emissions from cotton (*Gossypium hirsutum* L.) seedlings. *J. Chem. Ecol.* **20:** 3039–3050.

Mueller, T. F., and Phillips, J. R. (1983). Population dynamics of *Heliothis* spp. in spring weed hosts in southeastern Arkansas and stage-specific parasitism. *Environ. Entomol.* **12:** 1846–1850.

Puterka, G. J., Slosser, J. E., and Price, J. R. (1985). Parasites of *Heliothis* spp. (Lepidoptera: Noctuidae) parasitism and seasonal occurrence for host corps in the Texas Rolling Plains. *Environ. Entomol.* **14:** 441–446.

Röse, U. S. R., Manukian, A., Heath, R. R., and Tumlinson, J. H. (1996). Volatile semiochemicals released from undamaged cotton leaves: A systemic response of living plants to caterpillar damage. *Plant Physiol.* **111:** 487–495.

Röse, U. S. R., Lewis, W. J., and Tumlinson, J. H. (1997). Specificity of systemically released cotton plant volatiles as attractants for parasitic wasps (submitted for publication).

Stadelbacher, E. A., Powell, J. E., and King, E. G. (1984). Parasitism of *Heliothis zea* and *H. virescens* (Lepidoptera: Noctuidae) larvae in wild and cultivated host plants in the Delta of Mississippi. *Environ. Entomol.* **13:** 1167–172.

Steinberg, S., Dicke, M., and Vet, L. E. M. (1993). Relative importance of infochemicals from first to second trophic level in long-range host location by the larval parasitoid *Cotesia glomerata. J. Chem. Ecol.* **19:** 47–59.

Takabayashi, J., Dicke, M., and Posthumus, M. A. (1991). Variation in composition of predator-attracting allelochemicals emitted by herbivore-infested plants: relative influence of plant and herbivore. *Chemoecology* **2:** 1–6.

Turlings, T. C. J., Tumlinson, J. H., and Lewis, W. J. (1990). Exploitation of herbivore-induced plant odors by host seeking parasitic wasps. *Science* **250:** 1251–1253.

Turlings, T. C. J., Tumlinson, J. H., Heath, R. R., Proveaux, A. T., and Doolittle, R. E. (1991a). Isolation and identification of allelochemicals that attract the larval parasitoid *Cotesia marginiventris* to the microhabitat of its host. *J. Chem. Ecol.* **17:** 2235–2251.

Turlings, T. C. J., Tumlinson, J. H., Eller, F. J., and Lewis, W. J. (1991b). Larval-damaged plants: Source of volatile synomones that guide the parasitoid *Cotesia marginiventris* to the micro-habitat of its hosts. *Entomol. Exp. Appl.* **58:** 75–82.

Turlings, T. C. J., McCall, P. J., Alborn, H. T., and Tumlinson, J. H. (1993). An elicitor in caterpillar oral secretion that induces corn seedlings to emit chemical signals attractive to parasitic wasps. *J. Chem. Ecol.* **19:** 411–425.

Vet, L. E. M., Lewis, W. J., Papaj, D. R., and van Lenteren, J. C. (1990). A variable-response model for parasitoid foraging behavior. *J. Insect Behav.* **3:** 471–490.

Vinson, S. B. (1977). Behavioral chemicals in the augmentation of natural enemies. In Ridgeway, R. L., and Vinson, S. B. (Eds.), *Biological Control by Augmentation of Natural Enemies*, Plenum Press, New York, pp. 237–279.

Vinson, S. B., Henson, R. D., and Barfield, C. S. (1976). Ovipositional behavior of *Bracon mellitor* Say (Hymenoptera: Braconidae), a parasitoid of boll weevil (*Anthonomus grandis* Boh.). I. Isolation and identification of a synthetic releaser of ovipositor probing. *J. Chem. Ecol.* **2:** 431–440.

Wäckers, F. L., and Lewis, W. J. (1994). Olfactory and visual learning and their combined influence on host site location by the parasitoid *Microplitis croceipes* (Creson). *J. Biol. Control* **4:** 105–112.